Remarks

Claims 1, 3, 5, 9, and 12-14 are pending in this application.

Claims 1, 3, 5, 9, and 12-14 were rejected under 35 U.S.C. § 103(a) as being unpatentable over Lord (US 6,037,457) in view of Wilkins *et al.* (1992; "Wilkins"), Blomback *et al.* (1966; "Blomback"), Vukovich *et al.* (1980; "Vukovich"), Tripodi (WO 9213495), Garner *et al.* (US 5,639,940; "Garner"), Jennissen *et al.* (DE 4240119; "Jennissen"), Kutzko *et al.* (US 6,268,847; "Kutzko"), Sasaki *et al.* (US 4,295,855; "Sasaki"); and Clark *et al.* (US 5,935,850; "Clark"). Reconsideration is requested in view of the following remarks, and the Declaration of Henricus A. van Veen Under 37 C.F.R. 1.132. ("van Veen Decl."), submitted herewith.

The Examiner alleges that it would have been obvious to one of ordinary skill in the art to apply an isolation strategy similar to that of Lord et al (from cell culture) to isolate recombinant fibrinogen from milk by using the steps defined in pending claim 1 in view of the disclosures in Lord, Blomback, Wilkins, Tripodi and Vukovich. The Examiner alleges that the ordinary artisan would have been motivated to do so because the disclosures of the secondary references allegedly show that the strategy for isolating protein from plasma is equally applicable to the isolation of the same protein from milk. The Examiner alleges that the skilled person would have had a reasonable expectation that fibrinogen could be fractionally precipitated from milk before subjecting the precipitate to chromatography because this strategy was successful in the experiments described in Lord et al. Further, with regard to the use of HIC, the Examiner argues that the skilled person would seek to use an HIC resin in view of the particular disclosure in Jennissen, which employs HIC chromatography in a single purification step for fibrinogen from plasma. Thus, the Examiner concludes that the ordinarily skilled artisan would have had a reasonable expectation that a fibrinogen milk precipitate could be purified on HIC resin because purification is successful for fibrinogen from plasma.

Even though the asserted references may show precipitation as a purifying step for fibrinogen from milk, it is respectfully submitted that one of ordinary skill in the art would not be led to utilize HIC as defined to elute the high and low molecular weight sub fractions of fibrinogen to produce high Aα-chain integrity fibrinogen.

While HIC may have been used to purify fibrinogen, none of the documents relied upon by Examiner disclose possible separation of intact fibrinogen with high Aα-chain integrity from the other subfractions of fibrinogen that comprise Aα-chain degraded forms. Aα-chain degraded forms of fibrinogen occur as a result of the action of proteases in milk. This is discussed on page 30, lines 4-16 of the specification as filed and is furthermore discussed by Wilkins.

Human fibrinogen is present in three naturally occurring forms which differ in the degradation of the Aα-chain. These forms are non-degraded fibrinogen (HMW, 340 kDa, 70%), fibrinogen degraded at one Aα-chain (LMW, 300 kDa, 30%) and fibrinogen degraded at both Aα-chains (LMW', 280 kDa, 5%) (van Veen Decl. ¶4; specification, page 5, lines 4-16). The physiological process causing the degradation of the Aα-chain is as yet unknown; nevertheless, human fibrinogen purified from fresh plasma of healthy volunteers consists of these three forms which can be visualized by non-reduced SDS-PAGE (van Veen Decl. ¶4). On reduced SDS-PAGE, fibrinogen purified from fresh plasma provides Aα, Bβ and γ bands in a ratio of about 1:1:1 (van Veen Decl. ¶4). Taken together, these results indicate that reduced SDS-PAGE of freshly purified human fibrinogen does not discriminate between the three naturally occurring forms of fibrinogen whereas non-reduced SDS-PAGE does discriminate (van Veen Decl. ¶5). This finding is relevant to an interpretation of the results of Jennissen, a reference with Examiner alleges teaches HIC purification of undegraded fibrinogen.

Jennissen does not disclose and furthermore does not teach the method of the present invention in which fibrinogen having high Aα-chain integrity is obtained. Jennissen shows, on reduced SDS-PAGE, the recovery of the three chains of fibrinogen from plasma in a ratio of Aα, Bβ and γ of 1:1:1 (see column 5, lines 43-49 of the original German document, and page 6, lines 19-22 of the English translation). In view of the fact that reduced SDS-PAGE does not discriminate between the three naturally occurring variants of the fibrinogen Aα-chain, the results of Jennissen do not indicate separation of the naturally occurring Aα-chain degraded forms (van Veen Decl. ¶6). Further, Figure 1 of Jennissen shows an almost complete binding of fibrinogen from plasma to the pentyl Sepharose column which was eluted in only one peak. No additional peaks containing fibrinogen were observed that might indicate separation of the naturally occurring Aα-chain degraded forms of fibrinogen. Therefore, Jennissen does not

describe the elimination of Aα-chain degraded forms of fibrinogen (van Veen Decl. ¶6). There is no disclosure in Jennissen of the methods as presently defined in which HIC is used to obtain high Aα-chain integrity fibrinogen.

The selection of HIC for separation of intact high Aα-chain integrity (HMW) fibrinogen from Aα-chain degraded forms would not have been obvious to the person of ordinary skill in the art in view of the disclosure in Wolling *et al.*, *Berl. Munch Tierartz Wochenschr.* (1995); 108(11): 421-426, as discussed in the Amendment filed June 15, 2005. Wolling *et al.* failed to separate intact fibrinogen from degradation products using HIC. Indeed this reference discloses that HIC cannot be used for this purpose. The fact that HIC does in fact separate recombinant HMW (high Aα-chain integrity) fibrinogen from its Aα-chain degraded forms is surprising and indeed would not have been expected in view of the disclosure of Jennissen and the other prior art that has been asserted by Examiner.

The instant rejection is premised on the assumption that HIC performs identically as a purification medium for fibrinogen, regardless of the source of the fibrinogen. Examiner writes: "Thus the same precipitation and chromatography strategy for purifying fibrinogen from diverse sources it (sic) the same" (office action, p. 3). This is incorrect, in view of the following experiment reported by Mr. van Veen. Human fibrinogen obtained from fresh frozen plasma was subjected to HIC on Butyl Sepharose. The fibrinogen eluted as a single peak. Thus, the naturally circulating human variant forms of the Aα-chain (HMW, LMW and LMW') were not separated by HIC (van Veen Decl. ¶7). This is in contrast to the elution profile for purified recombinant human fibrinogen from transgenic bovine milk, which eluted in three peaks, the first of which represents the elution of high Aα-chain integrity fibrinogen (HMW fibrinogen) (van Veen Decl. ¶8).

In conclusion, the primary reference, Lord, does not teach a method as claimed for obtaining fibrinogen with high Aα chain integrity comprising an elution step which results in the selective removal of fibrinogen sub-fractions to produce high Aα chain integrity fibrinogen. Lord discusses the purification of fibrinogen from cell culture medium and <u>not</u> from milk. The deficiencies in Lord are not remedied by the secondary references. None of the secondary references teach or suggest using HIC to elute the high and low molecular weight sub fractions

of fibrinogen to produce high Aα-chain integrity fibrinogen. None of the references disclose possible separation of intact fibrinogen from its Aα-chain degraded forms. Moreover, the ability of HIC to separate out the Aα-chain degraded forms was unexpectedly found to be dependent on the source of the fibrinogen. HIC succeeded in separating the Aα-chain degraded forms from recombinant fibrinogen from transgenic bovine milk, but not human plasma fibrinogen. This is further evidence of the nonobviousness of the invention.

The claims remaining in the application are believed to be in condition for allowance. An early action toward that end is earnestly solicited.

Respectfully submitted

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Curriculum Vitae of Henricus Antonius ("Harrie") van Veen

Employment history

1991-present	Pharming Technologies B.V., Leiden, The Netherlands (Research and Development department)
1991-1997	Research Assistant, Protein Chemistry,
1997-2001	Research Associate, Protein Chemistry
2001-present	Scientist Process Development

Experience

- Developing purification processes for proteins using techniques commonly employed for protein fractionation such as precipitation, filtration and chromatography. The knowledge of filtration includes micro-, nano- and ultra-filtration in both dead-end as well as tangential flow. The chromatographic skills cover affinity-, ion-exchange-, hydrophobic interaction-, gel-filtration-, and reversed-phase chromatography including knowledge on chromatographic systems. Furthermore, production processes for biopharmaceuticals from milk of transgenic animals have been developed at lab-scale. These processes comply with the demands set by regulatory authorities.
- Experience with the development of analytical assays used for protein fractionation and protein characterization such as SDS/PAGE, immuno-assays (ELISA, RIA), and analytical chromatography.
- Outsourcing and transfer of developed purification processes.

Education

1981-1985	Junior general secondary education (MAVO) Don Bosco MAVO, Boskoop, The Netherlands
1985-1987	Senior general secondary education (HAVO) Samenwerkingsschool voor HAVO-VWO, Waddinxveen, The Netherlands
1987-1991	Higher laboratory education (HLO; BSc) Leidse Hogeschool, Leiden, The Netherlands Specialization: Biochemistry

Traineeship: Leiden Institute of Chemistry, Medical Biotechnology Department, Leiden University, headed by Prof. Dr. H.A. de Boer.
Project: Purification of human lactoferrin from the milk of transgenic mice, supervised by Dr. J.H. Nuijens.

Publications

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2. Appel MJ, van Veen HA, Vietsch H, Salaheddine M, Nuijens JH, Ziere B & de Loos F (2006) Sub-chronic (13-week) oral toxicity study in rats with recombinant human lactoferrin produced in the milk of transgenic cows, *Food Chem Toxicol* **44**, 964-973.

3. Thomassen EAJ, van Veen HA, van Berkel PHC, Nuijens JH & Abrahams JP (2005) The protein structure of recombinant human lactoferrin produced in the milk of transgenic cows closely matches the structure of human milk-derived lactoferrin, *Transgenic Research* **14**, 397-405.

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11. van Berkel PHC, Geerts MEJ, van Veen HA, Mericskay M, de Boer HA & Nuijens JH (1997) The N-terminal stretch Arg2 Arg3, Arg4 and Arg5 of human lactoferrin is essential for binding to heparin, bacterial lipopolysaccharide, human lysozyme and DNA, *Biochem J* **328**, 145-151.

12. van Berkel PH, van Veen HA, Geerts ME, de Boer HA & Nuijens JH (1996) Heterogeneity in utilization of N-glycosylation sites Asn624 and Asn138 in human lactoferrin: a study with glycosylation-site mutants, *Biochem J* **319**, 117-122.

13. van Berkel PHC, Geerts MEJ, van Veen HA, Kooiman PM, Pieper F, de Boer HA & Nuijens JH (1995) Glycosylated and unglycosylated human lactoferrins can both bind iron and have identical affinities towards human lysozyme and bacterial lipopolysaccharide, but differ in their susceptibility towards tryptic proteolysis, *Biochem J* **312**, 107-114.

14. Strijker R, Platenburg G, Nuijens J, Geerts M, van Veen H, Pronk A, Krimpenfort P, Eyestone W, Rademakers A, Pieper F, Kootwijk E, Kooiman P & de Boer H (1993) Production of human lactoferrin in milk of transgenic animals, *New Perspectives in Infant Nutrition (Renner, B and Sawatski, G, editors)* Thieme Medical Publishers Inc. NY, 115-119.

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Patents

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